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NITRIC OXIDE INHIBITION IN THE RAT IMPROVES BLOOD PRESSURE AND RENAL FUNCTION DURING HYPOVOLEMIC SHOCK

BY

W. LIEBERTHAL, A.E. McGARRY, J. SHEILS, AND C.R. VALERI

NAVAL BLOOD RESEARCH LABORATORY
BOSTON UNIVERSITY SCHOOL OF MEDICINE
615 ALBANY STREET
BOSTON, MA 02118

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We have examined the systemic and renal hemodynamic effects of nitric oxide (NO) inhibition with N^G-monomethyl-L-arginine (L-NMMA) in mormotensive rats as well as in rats with hypovolemic shock induced by hemorrhage. L-NMMA in-reased mean arterial blood pressure (MAP) from 114 ± 4 to 130 ± 6 mm Hg ($p < 0.05$) in the non-hemorrhaged rats and from 61 ± 3 to 89 ± 3 mm Hg ($p < 0.05$) in the hypovolemic animals. The absolute increase in MAP was greater in the hypovolemic (31 ± 3 mm Hg) than in the non-hemorrhaged

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(15 ± 2 mm Hg) rats ($p < 0.05$). An excess of L-arginine reversed the increase in MAP induced by L-NMMA in both groups. In the normotensive rats the increase in blood pressure was associated with an elevation in renal vascular resistance (RVR) (from 6.5 ± 0.7 to 8.2 ± 0.9 mm Hg/ml/min) ($p < 0.05$) so that renal plasma flow and GFR were unchanged. In contrast, in the hypotensive rats, the marked increase in MAP induced by L-NMMA infusion was not associated with a significant increase in RVR. As a result L-NMMA increased both renal plasma flow (from 6.0 ± 0.4 to 7.8 ± 0.4 ml/min) ($p < 0.05$) as well as GFR (from 1.7 ± 0.2 to 2.5 ± 0.2 ml/min) ($p < 0.05$). We conclude that NO is produced and modulates peripheral resistance in normotensive rats as well as in rats with hypovolemic shock. In the hypovolemic rats NO inhibition substantially improves renal plasma flow and GFR. Thus, NO production contributes to the hypotension and reduced function in hypovolemic rats by maintaining peripheral vasodilation.

ABSTRACT:

We have examined the systemic and renal hemodynamic effects of nitric oxide (NO) inhibition with N^G-monomethyl-L-arginine (L-NMMA) in normotensive rats as well as in rats with hypovolemic shock induced by hemorrhage. L-NMMA increased mean arterial blood pressure (MAP) from 114±4 to130±6mmHg ($p<0.05$) in the non-hemorrhaged rats and from 61±3 to 89±3mmHg ($p<0.05$) in the hypovolemic animals. The absolute increase in MAP was greater in the hypovolemic (31±3 mmHg) than in the non-hemorrhaged (15±2mmHg) rats ($p<0.05$). An excess of L-arginine reversed the increase in MAP induced by L-NMMA in both groups. In the normotensive rats the increase in blood pressure was associated with an elevation in renal vascular resistance (RVR) (from 6.5±0.7 to 8.2±0.9 mmHg/ml/min) ($p<0.05$) so that renal plasma flow and GFR were unchanged. In contrast, in the hypotensive rats, the marked increase in MAP induced by L-NMMA infusion was not associated with a significant increase in RVR. As a result L-NMMA increased both renal plasma flow (from 6.0±0.4 to 7.8±0.4ml/min)($p<0.05$) as well as GFR (from 1.7±0.2 to 2.5±0.2ml/min)($p<0.05$). We conclude that NO is produced and modulates peripheral resistance in normotensive rats as well as in rats with hypovolemic shock. In the hypovolemic rats NO inhibition substantially improves renal plasma flow and GFR. Thus, NO production contributes to the hypotension and reduced function in hypovolemic rats by maintaining peripheral vasodilation.

Index terms: nitric oxide, EDRF, N^G-monomethyl-L-arginine, hemorrhagic hypotension, blood pressure, renal hemodynamics

INTRODUCTION

Vascular endothelial cells produce nitric oxide (NO), a potent vasodilator, from the terminal guanidino nitrogen atom(s) of L-arginine (10,11). The synthesis of NO can be inhibited by analogues of L-arginine such as N^G-monomethyl-L-arginine (L-NMMA) (15). NO has been shown to account for many of the actions attributed to endothelium-derived relaxing factor (EDRF)(7,12). The role played by EDRF in mediating the vasorelaxant effects of a number of endothelium dependent vasodilators including acetylcholine, bradykinin and histamine was first described by Furchgott and his associates (5). These endothelium-dependent vasodilators have recently been shown to stimulate the production and release of nitric oxide (NO) from endothelial cells in amounts that account for the action of EDRF (7,12). Furthermore, inhibition of NO production prevents or blunts the vasodilation induced by acetylcholine in many vascular beds including the coronary (2) and renal circulations (17).

While NO is known to mediate the effects of endothelium-dependent vasodilators, the role of NO in modulating peripheral resistance and blood pressure *in vivo* remains uncertain. Recent studies using anologues of L-arginine such as L-NMMA have shown that inhibition of NO increases blood pressure in rats (17,18), rabbits (14) and guinea pigs (1). These data suggest that NO is produced *in vivo* and plays a role in the control of peripheral resistance by maintaining systemic vasodilation. Furthermore inhibitors of EDRF (9) and NO (13) have been shown to cause vasoconstriction in the isolated perfused kidney suggesting that NO modulates renal vascular resistance.

The role played by NO in hypotensive states has not been examined. Our objective was to determine whether NO contributes to the hypotension and

reduced renal perfusion in severely hypovolemic animals. In order to address this question, we have examined and compared the effects of L-NMMA on blood pressure and renal function in two groups: normotensive rats and rats with hypovolemic shock caused by acute hemorrhage. We have shown that NO inhibition increases blood pressure in both groups suggesting that NO is produced and modulates blood pressure in the hypovolemic animal as well as in the normotensive state. We have also shown that L-NMMA results in a substantial improvement in renal plasma flow and GFR in hypovolemic animals.

METHODS:

Male Sprague-Dawley rats weighing between 250 and 350 grams were used for all experiments. Rats were fed regular Purina rat chow (Ralston Purina, Chicago, IL) and allowed free access to water. Anesthesia was induced with an intraperitoneal injection of pentobarbital sodium (5mg/100g body weight and then maintained with a constant intravenous infusion of pentobarbital (91 ug/minute) throughout the study. Rats were placed on a thermostatically controlled, heated table. Body temperature was monitored with a rectal thermometer and maintained between 36°C and 38°C. A tracheostomy was performed using PE-240 tubing and the femoral artery was cannulated with PE-50 tubing for blood pressure monitoring as well as for blood sampling. A bladder catheter (PE-90) was placed via a suprapubic incision for urine sampling.

GFR was measured by inulin clearance using [*methoxy*-^{3}H]inulin (New England Nuclear, Boston, MA.) and effective renal plasma flow by para-aminohippurate (PAH) clearance using a chemical PAH assay (see assays). The left internal jugular vein was cannulated with two catheters of PE-50 tubing. PAH, inulin and pentobarbital, dissolved in 5% dextrose water, were infused via

one catheter at a rate of 0.028ml per minute. The other intravenous catheter was used for the infusion of either L-NMMA (Calbiochem, La Jolla, CA) or the L-NMMA vehicle. In those studies in which the effect of L-arginine was examined a third intravenous catheter was placed in the jugular vein for the infusion of L-arginine or its vehicle.

EXPERIMENTAL PROTOCOLS (Figure 1)

(1).Effect of L-NMMA in normotensive rats: After preparing the rats as outlined above, an infusion of inulin and PAH was begun. After an equilibration period of 30 minutes, a 20 minute baseline urine collection was obtained for measurement of inulin and PAH clearance. Blood pressure was monitored continuously throughout this period. Then an infusion of L-NMMA was begun and continued for 25 min. After five minutes of equilibration, a second 20 minute urine clearance period was obtained during which blood pressure was recorded and inulin and PAH clearances determined.

(2). Effect of L-NMMA during hemorrhagic hypotension. After obtaining a 20 minute baseline clearance period for inulin and PAH clearance determination and blood pressure monitoring, rats were subjected to hemorrhage. Whole blood (20ml/kg body weight) was removed through the femoral arterial catheter at a rate of 1 ml per minute. After a 45 min equilibration period a second 20 minute ("post-hemorrhage") clearance period was obtained for measurement of blood pressure, inulin and PAH clearance. Following this, the rats were divided randomly into two groups, an experimental and control group. In the experimental group an infusion of L-NMMA was begun while in controls the L-NMMA vehicle was given. Five minutes after starting L-NMMA or its vehicle, a third 20 min clearance period was obtained.

In an additional group of rats the effects of a prolonged infusion (one hour) of L-NMMA following hemorrhage was determined. After hemorrhage and a 45

minute equilibration period a "post-hemorrhage" clearance period was obtained as described above. Then an infusion of L-NMMA was begun. After a five minute equilibration period, three 20 minute clearance periods were obtained for determination of blood pressure and GFR.

3) Effects of L-arginine on blood pressure changes induced by L-NMMA

These protocols were carried out in normotensive rats as well as in rats made hypotensive by removal of 20ml/kg body weight as described above. In the normotensive rats the protocols were begun after a 30 minute equilibration period. In the hypotensive group the first period was started 45 minutes after hemorrhage.

Both normotensive and hypotensive rats were divided into three groups. In all, blood pressure was monitored during a control 20 minute baseline period. Then in groups 1 and 2, L-NMMA was infused. After a 5 minute equilibration period, blood pressure was recorded during a subsequent 10 min period. Then, while the infusion of L-NMMA continued, group 1 received L-arginine while group 2 received the vehicle for L-arginine. After another equilibration period of 15 min, blood pressure was again recorded during a third 10 minute period. In group 3 rats, blood pressure was recorded during a baseline period, during infusion of the L-NMMA vehicle and then during L-arginine infusion.

DOSES OF PHARMACOLOGIC AGENTS

L-NMMA. In preliminary studies the effect on blood pressure in both normotensive and hypotensive rats of four doses of L-NMMA was determined: 0.12, 0.24, 0.60 and 1.2 mg/kg/min. A maximum increase in blood pressure occurred at doses of 0.6 and 1.2mg/kg/min. In all further studies, L-NMMA was infused at 1.2mg/kg/min.

L-Arginine was infused at 12mg/kg/min

Vehicle: The vehicle for both L-NMMA and L-arginine was 5% dextrose water infused at 9uL/min.

ANALYTICAL METHODS.

Concentrations of [*methoxy*-^{3}H] inulin in urine and plasma was determined by liquid scintillation counting using a Beckman scintillation counter. Urine and plasma PAH concentrations were determined by chemical analysis as previously described (16).

CALCULATIONS.

1. GFR and PAH clearances were calculated with standard formulas.
2. Renal plasma flow (RPF) was calculated from the clearance of PAH assuming an extraction of 80% . (Renal extraction of PAH was measured in six rats and was the same before (78±5%) and during (79±5%) L-NMMA infusion.)
3. Filtration fraction (FF) was determined by dividing GFR by RPF.
4. Renal blood flow was calculated by dividing RPF by (1-hematocrit)
5. Renal vascular resistance (RVR) was calculated by dividing mean arterial blood presure (MAP) by renal blood flow.

STATISTICS.

All data are expressed as the mean±SE. All comparisons of two groups were made with the Student t test. All comparisons of more than two groups were made using analysis of variance (ANOVA) followed by the Scheffe test.
A p value <0.05 was considered significant.

RESULTS

(1) EFFECTS OF L-NMMA IN NORMOTENSIVE RATS (Table 1)(n=7).

L-NMMA increased blood presssure by 15±2mmHg in the normotensive animals (n=7) while GFR, renal plasma flow, renal blood flow and the filtration fraction were unchanged. RVR rose 26% during L-NMMA infusion .

(2) EFFECTS OF L-NMMA DURING HEMORRHAGIC HYPOTENSION

(Table 2)

Hemorrhage resulted in a comparable fall in MAP, RPF and GFR in the control and experimental groups. Subsequent infusion of the L-NMMA vehicle (in controls) did not alter any of these parameters. However infusion of L-NMMA during hypotension increased MAP (Figure 2) and RPF to levels that remained below baseline while GFR (Figure 2) was restored to values no different from baseline. The hematocrit fell with hemorrhage in both control and experimental rats However, hematocrit was not further altered by the subsequent infusion of L-NMMA vehicle or L-NMMA Neither the filtration fraction not RVR were altered by hemorrhage, by L-NMMA or by the L-NMMA vehicle.

In eight rats the effects of a prolonged (one hour) infusion of L-NMMA was studied. Blood pressure following hemorrhage was 55±5mmHg and rose to 95±5mmHg ($p<0.05$), 97±5mmHg ($p<0.05$) and 92±6mmHg ($p<0.05$) during the first, second and third 20 minute clearance periods obtained during L-NMMA infusion. GFR rose from 1.0±0.3ml/min during the "post hemorrhage" period to 2.2±0.2ml/min ($p<0.05$), 1.9±0.3ml/min ($p<0.05$) and 2.0±0.2ml/min ($p<0.05$) during the same three clearance periods obtained during L-NMMA. There was no difference in the blood pressure or GFR measurements among the three clearance periods obtained during L-NMMA infusion. Thus the effects of L-NMMA on blood presure and GFR were sustained in hypotensive animals.

(3) EFFECTS OF L-ARGININE ON BLOOD PRESSURE CHANGES INDUCED BY L-NMMA

NORMOTENSIVE RATS

Group 1(n=10): Blood pressure rose from 112±3mmHg to 128±5mmHg with L-NMMA and fell to baseline levels (116±4mmHg) with L-arginine (Figure 3)

Group 2(n=6): Blood pressure rose from 120±3mmHg to 133±4mmHg ($p<0.05$) with L-NMMA and remained elevated (140±4mmHg) ($p<0.05$ compared to baseline period) during infusion of L-arginine vehicle.

Group 3(n=6): Blood pressure during infusion of the L-NMMA vehicle (108±3mmHg) and the subsequent infusion of L-arginine (106±4mmHg) was not different from baseline levels (109±3mmHg).

HYPOTENSIVE RATS

Group 1(n=6): Blood pressure rose from 58±5mmHg to 96±7mmHg with L-NMMA and fell to baseline levels (71±8mmHg) with L-arginine (Figure 3)

Group 2(n=5): Blood pressure rose from 73±6mmHg to 99±7mmHg with L-NMMA ($p<0.05$) and remained elevated (95±7mmHg) ($p<0.05$ compared to baseline period) during infusion of L-arginine vehicle.

Group 3(n=6): Blood pressure during infusion of the L-NMMA vehicle (70±3mmHg) and subsequent infusion of L-arginine (74±4mmHg) was not different from baseline levels (63±4mmHg) .

DISCUSSION

We have shown that inhibition of NO production in the normotensive rat results in an increase in blood pressure (Table 1) that is reversed by an excess of L-arginine (Figure 3). These data suggest that NO is produced and modulates systemic vascular resistance and blood pressure in the

normotensive animal *in vivo* and confirms observations previously made by others (1,14,17,18)) . We have also examined the effects of L-NMMA when infused during hypotensive shock induced by acute hemorrhage. The infusion of L-NMMA following hemorrhage results in a striking increase in MAP (Table 2) which is reversed by L-arginine (Figure 3). This novel finding indicates that NO continues to be produced in the hypotensive animal and contributes to the reduced blood pressure by maintaining systemic vasodilation. While MAP is improved by L-NMMA it is not restored to the baseline, prehemorrhage value (Figure 2). The production of NO in both the normotensive and volume depleted rat does not appear to be limited by availability of precursor, since an excess of L-arginine has no effect on blood pressure in either situation when administered in the absence of L-NMMA.

The absolute increase in blood pressure induced by L-NMMA is substantially greater in the hypovolemic (31±3mmHg) than the normotensive rats (15±2mmHg) (Figure 4). The lesser blood pressure response to L-NMMA in the non-hemorrhaged rats group may be due to the activation of baroreceptor reflexes by the elevation of blood pressure into the hypertensive range with resultant inhibition of cardiac output. L-NMMA has previously been reported to cause reflex bradycardia normotensive rats (18). Another possible explanation for the observed difference in blood pressure response, is that NO production is enhanced in hypovolemic rats so that L-NMMA causes a greater degree of peripheral vasoconstriction than in non-hemorrhaged rats. Agonists such as vasopressin and nor-epinephrine, that are released during circulatory shock, have been shown to stimulate EDRF in isolated muscle strips of some species *in vitro* (6).

We have also examined the effect of NO inhibition on renal hemodynamics and function. In normotensive animals L-NMMA causes an increase in renal

vascular resistance while renal plasma flow and GFR remain unchanged (Table 1). In studies by other investigators, the effect of NO inhibition on GFR has been variable with some reporting no change (8) and others demontrating a significant fall in renal function (4) . The observed increase in renal resistance may be due to an autoregulatory response to the hypertension (3) , to direct inhibition of NO production within the kidney (9,13) or to a combination of these events.

In contrast to the effect in normotensive animals, GFR is markedly increased by L-NMMA in the hypovolemic rats (Figure 2). The increase in GFR appears to be due largely to the increase in RPF since filtration fraction is unchanged (Table 2) . However, in the absence of direct measurements of glomerular pressures and flows, minor changes in glomerular hydrostatic pressure or the glomerular ultrafiltration coefficient cannot be excluded.

The differences in the renal hemodynamic effects of L-NMMA in the normotensive and hypovolemic rats can be explained by the relative changes in MAP and RVR in the two groups. In the normotensive rats a 15% increase of MAP is matched by a 26% increase in RVR. In contrast, in the hypovolemic rats, L-NMMA causes a much greater increase in blood pressure (of 50%) but a statistically insignificant (18%) increase in RVR. Since L-NMMA increases MAP in the hypotensive rats without substantially increasing RVR, we infer that the peripheral vasoconstriction induced by L-NMMA occurs predominantly at vascular sites other than the kidney. The improvement in RPF and GFR during L-NMMA infusion in hypovolemic rats is therefore not due to a direct effect of NO inhibition on the renal circulation but is rather the indirect consequence of an increase in MAP in the absence of any substantial increase in RVR.

It is important to emphasize that the effects of L-NMMA observed in this study were obtained in anesthestized rats and that hemodynamic responses to NO inhibition may differ in conscious animals.

In summary, NO inhibition with L-NMMA increases blood pressure in the hypovolemic as well as the normotensive rat indicating that NO is produced and modulates peripheral resistance in both situations. In the normotensive rats, blood pressure is elevated to hypertensive levels by L-NMMA but the increased renal perfusion pressure is associated with an elevation in renal vascular resistance so that renal perfusate flow and GFR remain unchanged. In hypotensive rats L-NMMA results in a dramatic improvement in blood pressure but causes little change in renal vascular resistance. As a result there is a substantial increase in renal plasma flow rate and a consequent restoration of GFR. We conclude that the production of NO contributes to the hypotension and renal hypoperfusion in severely hypovolemic rats.

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TABLE 1

Effects of L-NMMA on systemic and renal hemodynamics in non-hemorrhaged rats.

	Baseline	During L-NMMA
MAP (mmHg)	114±4	130±6*
GFR (ml/min)	2.5±0.1	2.4±0.2
RPF (ml/min)	10.0±1.0	9.5±1.1
FF (%)	26±2	26±2
RVR (mmHg/ml/min)	6.5±0.7	8.2±0.9*

Values expressed as mean±SE (n=7)
MAP=mean arterial pressure;RPF=renal plasma flow;GFR=glomerular filtration rate;
FF=filtration fraction; RVR=renal vascular resistance

*$p<0.05$ L-NMMA period compared to baseline period

TABLE 2

Systemic and renal hemodynamic effects of L-NMMA or the L-NMMA vehicle following hypotensive hemorrhage.

	CONTROL GROUP (n=9)			EXPERIMENTAL GROUP (n=8)		
	Baseline period	Post-hemorrhage	L-NMMA vehicle	Baseline period	Post-hemorrhage	L-NMMA
MAP (mmHg)	108±3	68±4*	68±2*	111±2	61±3*	89±3*†
RPF (ml/min)	10.5±1.1	6.9±0.8*	6.7±0.6*	10.4±0.5	6.0±0.4*	7.8±0.4*†
GFR (ml/min)	2.8±0.2	1.7±0.2*	1.9±0.1*	2.8±0.1	1.7±0.2*	2.5±0.2†
FF (%)	28±3	26±2	29±2	28±1	28±2	32±2
Hematocrit (%)	48±1	39±1*	39±1*	45±1	38±1*	39±1*
RVR (mmHg/ml/min)	5.9±0.6	6.2±0.4	6.6±0.7	6.0±0.3	6.4±0.5	7.1±0.5

*$p<0.05$ compared to baseline period and †$p<0.05$ compared to post-hemorrhage period within the same group.
Control group received the L-NMMA vehicle (dextrose water at 9ul/min) after hemmorrhage
Experimental group received L-NMMA (1.2mg/kg/min) following hemorrhage

FIGURE LEGENDS

Figure 1

Experimental protocols (see text for details)

Figure 2

Effect of L-NMMA on mean arterial blood pressure (upper panel) and GFR (lower panel) following hemorrhagic hypotension. Experimental rats (closed squares) were given L-NMMA following hemorrhage, while controls (open squares) were given the L-NMMA vehicle.

All values expressed as mean±SE

*$p<0.05$ compared to baseline value (for both experimental and control groups)

†$p<0.05$ compared to post hemorrhage period

Figure 3

Effect of L-arginine on blood pressure changes induced during infusion of L-NMMA in normotensive rats (represented by open squares)(n=10) and hypotensive rats (represented by closed squares)(n= 6).

All values expressed as mean ±SE

*$p<0.05$ L-NMMA period compared to baseline value.

†$p<0.05$ L-arginine+L-NMMA period compard to L-NMMA period.

Figure 4

Comparison of the absolute increase in mean blood pressure (MAP) induced by L-NMMA in normovolemic rats (n=25) and in hypovolemic rats (n= 19).

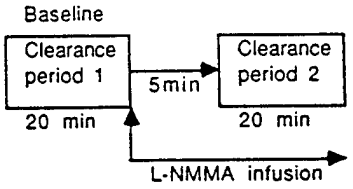
All values expressed as mean ±SE

*$p<0.05$ hypotensive group compared to normotensive group

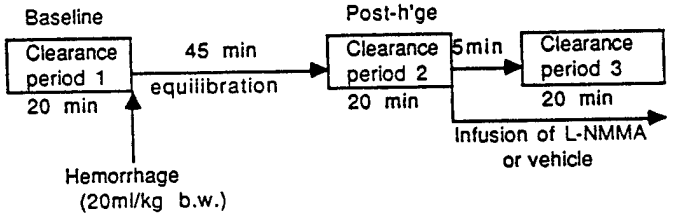
FIGURE 1

EXPERIMENTAL PROTOCOLS

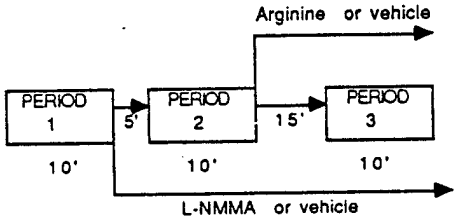
1.Effect of L-NMMA in normotensive rats



2. Effect of L-NMMA following hemorrhage



3. Effects of L-arginine on blood pressure changes induced by L-NMMA



Groups studied:
- Group 1: L-NMMA followed by arginine
- Group 2: L-NMMA followed by arginine vehicle
- Group 3: L-NMMA vehicle followed by arginine.

FIGURE 2

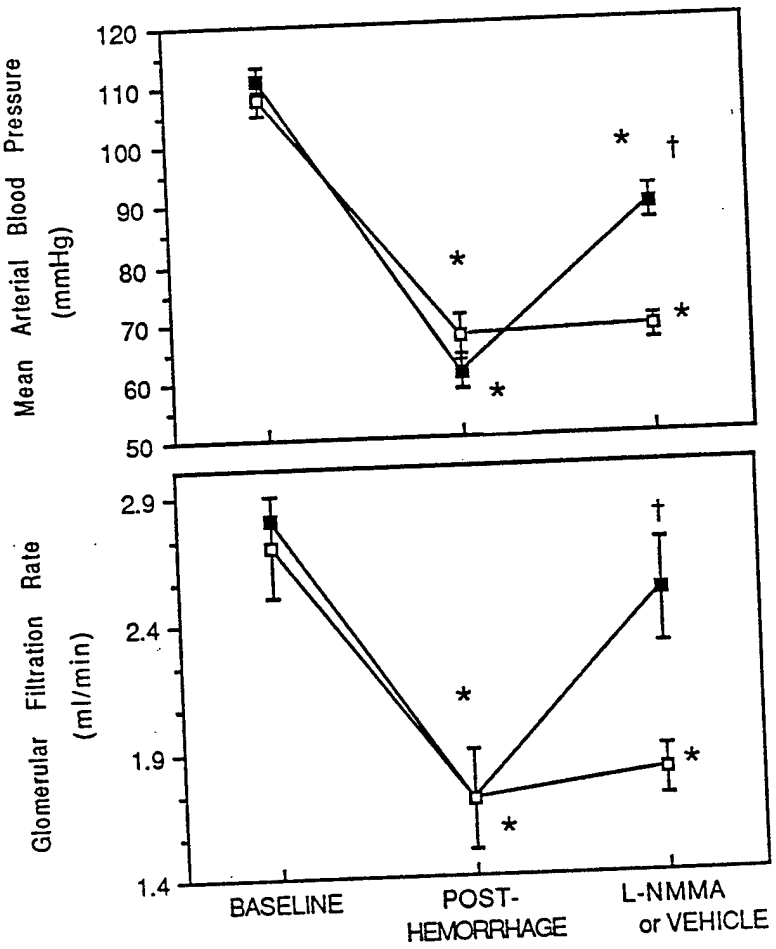

Mean Arterial Blood Pressure (mmHg)
120
110
100
90
80
70
60
50
Glomerular Filtration Rate (ml/min)
2.9
2.4
1.9
1.4
*
†
BASELINE
POST-HEMORRHAGE
L-NMMA or VEHICLE

FIGURE 3

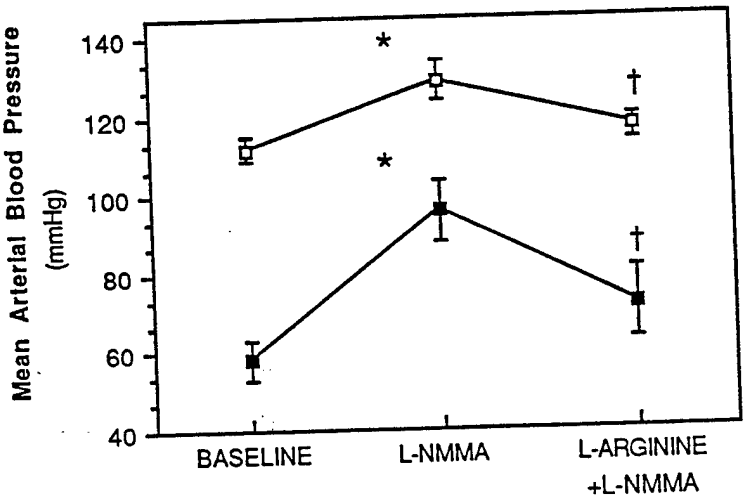
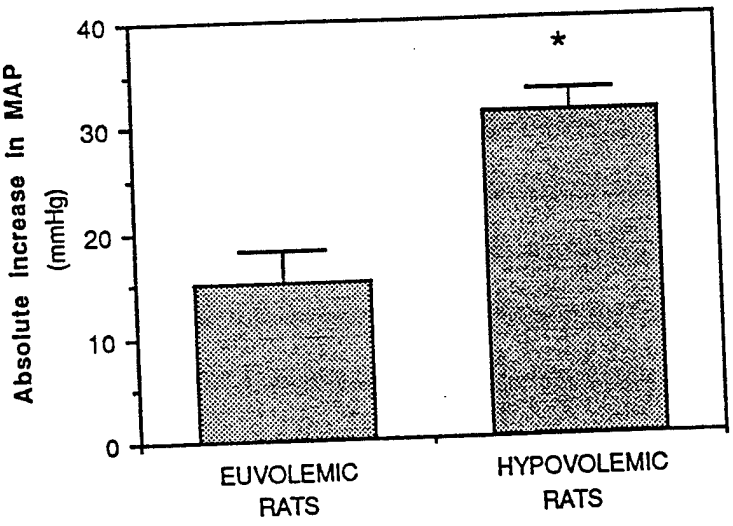

Mean Arterial Blood Pressure
(mmHg)
140
120
100
80
60
40
*
*
†
†
BASELINE
L-NMMA
L-ARGININE
+L-NMMA

FIGURE 4


Absolute Increase In MAP
(mmHg)
40
30
20
10
0
*
EUVOLEMIC
RATS
HYPOVOLEMIC
RATS